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Mycorrhizal symbiosis affects ABA metabolism during berry ripening in *Vitis vinifera* L. cv. Tempranillo grown under climate change scenarios

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ABSTRACT

Arbuscular mycorrhizal symbiosis is a promising tool for improving the quality of grapes under changing environments. Therefore, the aim of this research was to determine if the ability of arbuscular mycorrhizal fungi (AMF) to enhance phenolic content (specifically, anthocyanins) in a climate change framework could be mediated by alterations in berry ABA metabolism during ripening. The study was carried out on fruit-bearing cuttings of cv. Tempranillo (CL-1048 and CL-1089) inoculated (+M) or not (−M) with AMF. Two experimental designs were implemented. In the first experiment +M and -M plants were subjected to two temperatures (24/14 °C or 28/18 °C (day/night)) from fruit set to berry maturity. In the second experiment, +M and -M plants were subjected to two temperatures (24/14 °C or 28/18 °C (day/night)) combined with two irrigation regimes (late water deficit (LD) and full irrigation (FI)). At 28/18 °C AMF contributed to an increase in berry anthocyanins and modulated ABA metabolism, leading to higher ABA-GE and 7′OH-ABA and lower phaseic acid (PA) in berries compared to –M plants. Under the most stressful scenario (LD and 28/18 °C), at harvest +M plants exhibited higher berry anthocyanins and 7´OH-ABA and lower PA and dihydrophaseic acid (DPA) levels than –M plants. These findings highlight the involvement of ABA metabolism into the ability of AMF to improve some traits involved in the quality of grapes under global warming scenarios.

1. Introduction

Global warming is expected to reduce food production in the future. Viticulture is one of those sectors most sensitive to both short- and long-term climate changes due to the narrow cultivation niches of vines [1]. Climate scenarios for South Mediterranean Europe predict an increase in temperature, alterations in rainfall patterns and an increasing frequency of extreme climate events, all of which will negatively affect viticulture in the region [2,3]. In spite of accounting for 14% of the surface area of vineyards in the world, the Spanish surface area has fallen from 1196 kha in 1995 to 975 kha in 2016 and in this context future projections are unlikely to be positive without adaptation. Almost 90% of the total Spanish grape production is used to produce wine, with a production of 33.5 Mh L in 2017, which represents a reduction of 5.8% with respect to 2016 [4]. Among red wine varieties, Tempranillo is one of the dominant varieties in Spain, where it accounts for 21% of the total Spanish vineyard surface [4]. The Tempranillo variety is characterized by early ripening with a short vegetative cycle. Although these traits are not relevant for coping with climate change, it is difficult to change the established grapevine cultivars in a specific region because of the narrow dependency on consumer preferences which are often linked to a certain particular wine taste [5]. In this context, producers and markets are clearly aware of the risks and opportunities of climate change and demand information on future choices [6].

Climate change affects winemaking because it reduces grapevine yields and modifies berry composition due to its great impact on berry growth and ripening [7]. Among the most important warming-related effects on grapevines are the significant advance in phenology (i.e., budburst, flowering and veraison dates) [8,9], increases in berry sugar concentrations that lead to high wine alcohol levels, lower acidity levels, delays in the synthesis of phenolic compounds [10] and changes in berry skin metabolite profiles [11–13]. In addition to temperature, the predicted reductions in rainfall imply that vines may require supplemental irrigation to limit water deficit stress during the grapevine growing season [14]. As a result, different irrigation programs have been implemented in South Mediterranean areas, which allow the control of vegetative development and reduction in berry size,

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improvement of the cluster microclimate, increases in water use efficiency, and the enhancement of the sugar and phenolic content of berries [15–18].

Within the climate change scenario, new strategies are crucial to maintaining grape quality under the future environmental constraints. Maintaining soil quality in order to improve the beneficial relationships between plants and arbuscular mycorrhizal fungi (AMF) could be a suitable option. Considerable progress has been made in the last decade in the use of AMF to improve plant growth and yield for crop plants in general, but also for grapevines, particularly as AMF symbiosis plays a major role in the survival of grapevines in their natural habitats [19]. Thus, the adaptation of viticulture to climate change may benefit from AMF since root colonization increases grapevine growth and nutrition, tolerance to abiotic stresses, protects against biotic stresses, and increases soil stability [20]. Furthermore, AMF colonization induces changes in plant secondary metabolism leading to enhanced biosynthesis of polyphenols, carotenoids or flavonoids [21–25].

Phytohormone abscisic acid (ABA) has been considered as the main mediator of grapevine response to abiotic stress, such as water deficit [26] or elevated temperature [27,28]. Furthermore, ABA plays a crucial role in grape berry development and ripening [29,30]. Thus, the accumulation of ABA around veraison is accompanied by sugar accumulation, colour development, and berry softening, suggesting that ABA may play a major role in controlling several ripening-associated processes [30–33]. Nevertheless, a lack of correlation between free-ABA in berries and 9-*cis*-epoxycarotenoid dioxygenase (NCED), the enzyme involved in the first step of ABA synthesis suggests that compounds derived from ABA catabolism/conjugation could also be involved in berry ripening [32,34]. Active ABA can be metabolized in a variety of ways [35]. It can be conjugated to glucose forming the inactive ABA-glucose ester (ABA-GE), which is stored or transported [36] or alternatively, ABA can be catabolized by hydroxylation at the positions 7′, 8′ and 9′. Hydroxylation at the 7′ position produces 7-hydroxy-ABA (7′OH-ABA) and at the 8′ position phaseic acid (PA) and subsequently dihydrophaseic acid (DPA). Recent research has shown that ABA concentrations and catabolites were also regulated by the intensity and/or timing of water deficit [31,37,38] and temperature [39].

Plant hormones also interact to regulate the establishment and functioning of symbiotic associations with AMF [40]. Specifically, it has been demonstrated that ABA is essential for root colonization and for the functionality of the fungal structures [41]. In view of the role of ABA in the regulation of some berry ripening processes, and given that ABA concentration is enhanced by AMF in leaves, especially under abiotic stress [42,43], it is of interest to investigate the possible role of AMF inoculation on the levels of free ABA and its catabolites throughout berry ripening. Therefore, the aim of this research was to determine if the ability of AMF for inducing the accumulation of anthocyanins in grapes under a climate change framework could be mediated by alterations in the metabolism of ABA during berry ripening. Previous research has demonstrated that fruit-bearing cuttings are a useful model system to study the response of berry ripening to environmental factors [24,25,44–46]. Hence, potted vines were used to control mycorrhizal inoculation and to have comparable non-inoculated plants.

2. Material and methods

2.1. Plant material and growth conditions

Vitis vinifera (L.) cuttings from different clones of Tempranillo were obtained from an experimental vineyard of the Institute of Sciences of Vine and Wine (Logroño, Spain) (Denomination of Origin Rioja, North of Spain) during the winter. The study was performed in two clones (CL) of different origins (CL-1048, from Laguardia (Álava), and CL-1089, from Bargota (Navarra)) that were selected in the field on the basis on their different agronomic traits and plant material availability. Both clones have a short reproductive cycle but differed in yield, which was medium for CL-1048 and high for CL-1089). Dormant 400–500 mm long cuttings of each clone were selected for fruit-bearing according to the steps originally outlined by Mullins [47] with slight modifications as described in Ollat et al. [48] and Antolín et al. [49]. Briefly, rooting was induced with indole butyric acid (400 mg L^{-1}) in a heat-bed (27 °C) kept in a cold room (4 °C). Once cuttings had developed roots, they were transplanted to 6.5-L plastic pots containing a mixture of vermiculite–sand–light peat (2.5:2.5:1, v:v:v). The properties of the peat (Floragard, Vilassar de Mar, Barcelona, Spain) were pH 5.2–6.0, nitrogen 70–150 mg L^{-1}, P_2O_5 80–180 mg L^{-1}, and K_2O 140–220 mg L^{-1}. The peat was previously sterilised at 100 °C for 1 h on 3 consecutive days.

At transplantation, the fruit-bearing cuttings were transferred to two growth chamber-greenhouses (GCG) adapted to provide different climate change scenarios [50] until berry maturity was reached. In both GCG, initial growth conditions were a 25/15 °C and 50/90% relative humidity (day/night) regime and natural daylight (photosynthetic photon flux density, PPFD, was on average 850 µmol m^{-2} s^{-1} at midday) supplemented with high-pressure sodium lamps (SON-T Agro Phillips, Eindhoven, Netherlands) to extend the photoperiod up to 15 h and ensure a minimum PPFD of 350 µmol m^{-2} s^{-1} at the level of the inflorescence. Humidity and temperature were controlled using M22W2HT4X transmitters (Rotronic Instrument Corp., Hauppauge, USA). PPFD was monitored with a LI-190SZ quantum sensor (LI − COR, Lincoln, USA). Under these conditions, plants reached bud-break one week later. In order to improve the partitioning of stored carbon towards the roots and the reproductive structures, vegetative growth before flowering was controlled carefully. Thus, only a single flowering stem was allowed to develop on each plant during growth. Until fruit set, plants were watered twice a day with a nutrient solution (140 mL day^{-1}) with a phosphorus level of 0.30 mM [48] alternated with water. The electric conductivity of the nutrient solution adjusted to pH 5.5 was 1.46 ± 0.15 mS cm^{-1} as determined with a conductivity meter (524; Crison Instruments SA, Alella, Spain). Under these conditions, fruit set (Eichhorn and Lorenz (E–L) growth stage 27) [51] took place 30 days after bud break and plants had 4-5- fully expanded leaves.

2.2. Mycorrhizal inoculation

At transplantation, half of the plants were inoculated with the commercial mycorrhizal inoculum Bioradis Gel (Bioera SLU, Tarragona, Spain) (+M plants). The inoculum consisted of a mixture of five AMF fungi (*Septglomus deserticola*, *Funneliformis mosseae, Rhizoglomus intraradices, Rhizoglomus clarum* and *Glomus aggregatum*), containing 100 spores per g of inoculum and a mixture of rhizobacteria belonging to the *Bacillus* and *Paenibacillus* genera (2×10^6 cfu g^{-1}). The microbial preparation was diluted in distilled water (1:20) so that each plant received 1 g of product. The roots of +M fruit bearing cuttings were submerged in the gel for 15 min, and then plants were placed in the pots. In order to discriminate the effects on plant metabolism due to the action of the mycorrhizal symbiosis, half of the plants were kept as non-inoculated controls. Uninoculated plants (M plants) were submerged directly for 15 min in the filtrate of mycorrhizal inocula with the objective of restoring rhizobacteria and other soil free-living microorganisms accompanying AMF and which play an important role in the uptake of soil resources as well as in the infectivity and efficiency of AMF isolates [52]. The filtrate was obtained by passing diluted mycorrhizal inoculum through a layer of 15-20-mm filter paper with particle retention of 2.5 mm (Whatman 42; GE Healthcare, Little Chalfont, UK). All plants (-M and + M) were fertilized as described previously. Studies carried out by our group [24] had demonstrated that a phosphorus level of 0.30 mM was sufficient to ensure an adequate development of -M plants, even under water deficit [25], and not too high to impede the correct establishment of the mycorrhizal symbiosis.

2.3. Experimental design

Two separate experimental designs were performed to assess the grapevine responses under different climate change scenarios. In a first experiment (experiment 1), we chose Tempranillo CL-1048 because previous data had shown that AMF inoculation improved berry properties in this clone when subjected to elevated temperatures [24]. Fruit-bearing cuttings from + M or -M treatments were divided into two GCG to be exposed to each temperature regime: 24/14 °C (day/night) and 28/18 °C (day/night) from fruit set (E–L 27 stage) to berry maturity (E–L 38 stage). The 24/14 °C temperature regime was selected according to average temperatures recorded in La Rioja (1981–2010) [53] during the growing season. The 28/18 °C temperature regime was selected according to predictions of a rise of 4.0 °C at the end of the present century [54]. To avoid excessive soil overwarming, which can negatively affect roots, and maintain a stable temperature, pots were shaded by wrapping their lateral surface with a reflecting material. Soil temperature was monitored at a depth of 5 cm soil using temperature probes PT100 (Coreterm, Valencia, Spain) and reached 24 ± 0.5 °C and 28 ± 0.5 °C for 24/14 °C and 28/18 °C air temperature regimes, respectively. Berry samples were collected at five stages of berry development: 1) when berries began to soften (Eichhorn and Lorenz (E–L) growth stage 34, green berries); 2) when berries began to colour and enlarge (E–L 35 stage, mid-veraison); 3) one week after mid-veraison (E–L 36 stage); 4) two weeks after mid-veraison (E–L 37 stage); and 5) at commercial maturity (22°Brix) (E–L 38 stage).

In a second experiment (experiment 2), we chose the clone CL-1089 because previous data had shown that AMF inoculation improved anthocyanin accumulation under deficit irrigation and elevated temperatures [25]. Thus, we established a three-factorial design where the two temperature regimes (24/14 °C and 28/18 °C) were combined with two water regimes. Within each temperature regime, fruit-bearing cuttings of CL-1089 from + M or -M treatments were divided into two groups: 1) plants under full irrigation (FI) from fruit set (E–L 27 stage) to maturity (E–L 38 stage) and 2) plants that received 50% of the water given to FI plants from veraison (E–L 35 stage) to maturity (E–L 38 stage) (late deficit, LD). Until the beginning of treatment, LD plants were maintained to full irrigation. Soil moisture sensors (EC-5 Soil Moisture Sensors, Decagon Devices Inc., Pullman, WA, USA) were placed in the pots. FI plants were maintained at ca. 80% of pot capacity (sensor value between 40 and 50%, (m^3 H_2O m^{-3} soil) × 100) until fruit harvest. Pot capacity was previously assessed by determining water retained after free-draining water had been allowed to pass through the holes in the bottom of the pot. The surface of the plant containers was covered with quartz stones during the experiments to avoid water loss because of evaporation. The water volume supplied to the FI treatment was adjusted to increase plant development according to the daily measurements of the EC 5 water sensor. Watering was performed with nutrient solution or deionised water in order to supply the different treatments with the same amount of nutrients during water deficit. Predawn leaf water potential (Ψ_{pd}) was measured with a SKYE SKPM 1400 pressure chamber (Skye Instruments Ltd, Llandrindod, Wales, UK) on three fully expanded leaves per treatment at each sampling date just prior to irrigation (Fig. S1). In this experiment, berry samples were collected at three stages of berry development: 1) one week after mid-veraison (E–L 36 stage); 2) two weeks after mid-veraison (E–L 37 stage); and 3) at commercial maturity (22°Brix) (E–L 38 stage).

2.4. Mycorrhizal colonization

Root samples were cleared and stained following the procedure described in Koske and Gemma [55]. A potassium hydroxide solution (10% w:v) was added to the roots which were placed in an oven at 70 °C for 2 h. After being rinsed with water, roots were clarified by the addition of H_2O_2 (3% v:v) and subsequent washing with water. Then, they were acidified by soaking in HCl (1% v:v) for 5–15 minutes and stained in a solution of methyl blue: lactic acid (1% w:v) at 70 °C for 1 h. Stained roots were stored in a mixture of glycerol, water and HCl 1% (500:450:50, v:v:v) until quantification. The percentage of mycorrhizal colonization was determined under a stereoscopic microscope by the plate intersection method [56].

2.5. Berry determinations

For each stage, berries were collected and frozen in liquid nitrogen and kept at -80 °C until determinations. When fruit maturity was reached, plants were harvested separately based on sugar level from berry subsamples (2–3 berries) taken weekly. The length of phenological phases was recorded as the number of days from fruit set (E–L 27 stage) to each of the abovementioned berry stages.

A subsample of 5 berries was crushed and then extracts were centrifuged at 4300 *g* at 4 °C for 10 min. The supernatant was used for determination of total soluble solids (mainly sugars) measured with a temperature-compensating refractometer (Zuzi model 315; Auxilab, Beriáin, Spain) and expressed as °Brix. Another subsample of 5 berries was taken for the analysis of anthocyanins, total phenols and abscisic acid (ABA) metabolites. Berries were ground separately to a powder in a mortar with liquid nitrogen and weighed. Anthocyanins were calculated according to the procedure described by Saint-Cricq et al. [57]. Two samples of the non-filtered, crushed grape homogenate were macerated for 4 h at pH 1 (hydrogen chloride) and pH 3.2 (tartaric acid), respectively. Once maceration was over, the macerated samples were centrifuged at 4300 *g* at 4 °C for 10 min. Total anthocyanins were determined in supernatant (macerated at pH 1) according to Ribéreau-Gayon and Stonestreet [58] by reading absorbance at 520 nm. Calibration was performed by using malvidin-3-glucoside as a standard and anthocyanins were expressed as mg g^{-1} DM. Phenolic substances were estimated by reading absorbance at 280 nm in the supernatant obtained after maceration at pH 3.2 and results were expressed as gallic acid equivalent (mg g^{-1} DM) [59]. All analyses were run in triplicate.

2.6. ABA and catabolite analyses

The extraction, purification, and quantification of abscisic acid (ABA) and its catabolites (abscisic acid glucosylester (ABA-GE), 7-hydroxyl-ABA (7′OH-ABA), dihydrophaseic acid (DPA) and phaseic acid (PA)) were carried out in 0.1 *g* of the frozen powdered material as recently described by Chini et al. [60] with some modifications. Briefly, 1 mL of precooled (-20 °C) methanol:water:formic acid (90:9:1, v/v/v with 2.5 mM Na-diethyldithiocarbamate) and 10 µL of deuterium labelled internal standards [([$^2H_4$]-ABA), ([$^2H_5$]-ABA-GE), ([$^2H_3$]-DPA), ([$^2H_3$]-PA) and ([$^2H_4$]-7 − OH-ABA) provided by The National Research Council of Canada, Saskatoon, Saskatchewan, Canada) in methanol, were added to each sample. After shaking in a Multi Reax shaker (Heidolph Instruments) at 2000 r.p.m. for 60 min at room temperature, solids were separated by centrifugation at 20,000 *g* for 10 min at room temperature in a Sigma 4–16 K Centrifuge (Sigma Laborzentrifugen), and re-extracted with an additional 0.5 mL extraction mixture, followed by shaking (20 min) and centrifugation. 1 mL of the pooled supernatants was separated and evaporated at 40 °C using a RapidVap Evaporator (Labconco Co). The residue was redissolved in 0.5 mL of methanol: 0.133% acetic acid (40:60, v/v). The solution was centrifuged at 20,000 *g* for 10 min at room temperature before injection into the high resolution accurate mass spectrometry (HPLC-ESI-HRMS) system.

The quantification was carried out using a Dionex Ultimate 3000 UHPLC device coupled to a Q Exactive Focus Mass Spectrometer (Thermo Fisher Scientific) equipped with an HESI(II) source, a quadrupole mass filter, a C-trap, a HCD collision cell and an Orbitrap mass analyser, using a reverse-phase column (Synergi 4 mm Hydro-RP 80 A, 150 × 2 mm; Phenomenex). A linear gradient of methanol (A), water (B) and 2% acetic acid in water (C) was used: 38% A for 3 min, 38% to

Table 1
Experiment 1: percentage of mycorrhizal colonization and total phenolics recorded at the harvest of fruit-bearing cuttings of Tempranillo (CL-1048) inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M) and grown at 24/14 °C (24) or 28/18 °C (28) (day/night) temperatures (T).

Treatments	Mycorrhizal colonization (%)	Soluble phenolic substances (mg g^{-1} DM)
Treatments		
-M24	–	40.4
+M24	45.3 b	42.9
-M28	–	32.6
+M28	62.3 a	29.4
Main effects		
Temperature (T)		
24	–	41.7 a
28	–	31.0 b
Mycorrhization (M)		
-M	–	36.5
+M	–	36.2
ANOVA		
T × M	ns	ns

Values represent means (n = 4) separated by Duncan's test (at $P \leq 0.05$). Within columns, means followed by different letters are significantly different as affected by the main factors temperature (24, 28), mycorrhization (+M, –M) and their interaction. ns, not significant ($P > 0.05$). DM: dry matter.

96% A in 12 min, 96% A for 2 min, and 96% to 38% A in 1 min, followed by stabilization for 4 min. The percentage of C remained constant at 4%. The flow rate was 0.30 mL min^{-1}, injection volume was 40 µL, and column and sample temperatures were 35 and 15 °C, respectively. Ionization source working parameters were optimized (Table S1). The detection and quantification were performed by a full MS experiment with MS/MS confirmation in the negative-ion mode, employing multilevel calibration curves with deuterated hormones as internal standards. MS1 extracted from the full MS spectrum was used for quantitative analysis and MS2 for confirmation of target identity. For full MS, a *m/z* scan range from 62 to 550 was selected, resolution was set at 70,000 full width at half maximum (FWHM), automatic gain control (AGC) target at $1e^6$ and maximum injection time (IT) at 250 ms. A mass tolerance of 5 ppm was accepted. The MS/MS confirmation parameters were resolution of 17,500 FWHM, an isolation window of 3.0 *m/z*, AGC target of $2e^5$, maximum IT of 60 ms, loop count of 1 and minimum AGC target of $3e^3$. Instrument control and data processing were carried out with TraceFinder 3.3 EFS software. Accurate masses of ABA, its metabolites and internal standard, as well as their principal fragments are reported in Table S2 and a chromatographic profile of ABA and its metabolites is shown in Fig. S2.

2.7. Statistical analysis

Statistical analyses were carried out using statistical software the Statistical Package for the Social Sciences (SPSS) (SPSS Inc., Chicago, IL, USA) version 21.0 for Windows. Data were subjected to Kolmogorov-Smirnov normality test due to the small sample size (n = 4). Data appeared to follow a normal distribution and were thus subjected to analysis of variance (ANOVA). In the first experiment, tests were performed to assess the main effect of the factors temperature (T) (24/14 °C, 24 and 28/18 °C, 28), AMF inoculation (+M and –M) and the interaction between these factors. In the second experiment, tests evaluated the main effect of the factors temperature (T) (24/14 °C, 24 and 28/18 °C, 28), AMF inoculation (+M and –M) and irrigation program (FI and LD) and the interaction between them. Means ± standard errors (SE) were calculated and when the F ratio was significant ($P \leq 0.05$), a Duncan test was applied. Two-way or three-way ANOVAs were performed to determine significant differences in all measured parameters in CL-1048 or CL-1089, respectively.

3. Results and discussion

Previous research has shown the ability of Tempranillo grapevine to adapt to different environmental constraints associated with climate change, such as elevated air temperature and water deficit, both of which ultimately benefited berry properties [13]. In this context of heat and drought, AMF inoculation has been shown to be an appropriate resource to maintain or improve Tempranillo berry quality [24,25]. For these reasons, a detailed study into berry ABA metabolism was performed to explore the mechanism underlying this effect.

	Main effects				ANOVA
°Brix	24	28	-M	+M	T × M
E-L34	7.0	7.1	7.1	6.9	ns
E-L35	10.0	10.3	10.2	10.1	ns
E-L36	14.2	15.2	13.8	15.3	ns
E-L37	17.5	19.6	18.6	18.4	ns
E-L38	21.5	22.5	21.8	22.2	*

	Main effects				ANOVA
Days	24	28	-M	+M	T × M
E-L34	54.3 a	41.4 b	47.8	47.9	ns
E-L35	65.4 a	49.9 b	56.6	58.6	ns
E-L36	72.4 a	56.9 b	63.6	65.6	ns
E-L37	79.4 a	63.9 b	70.6	72.6	ns
E-L38	110.6 a	83.1 b	94.1	99.6	ns

Fig. 1. Experiment 1: evolution of total soluble solids (°Brix) recorded during berry ripening in fruit-bearing cuttings of Tempranillo (CL-1048) inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M) and grown at 24/14 °C (24) or 28/18 °C (28) (day/night) temperatures. Values represent means ± SE (n = 4). A two-way ANOVA analysis was performed to evaluate the effects of temperature (T), mycorrhizal inoculation (M) and their interaction. ns, and * indicate non-significance or significance at 5% probability levels, respectively. When interaction between the main factors 'temperature, T' and 'mycorrhizal inoculation, M' was significant, different letters indicate significant differences according to Duncan test ($P \leq 0.05$).

	Main effects				ANOVA
	24	28	-M	+M	T × M
E-L34	0.54	0.98	0.80	0.73	**
E-L35	3.49	5.07	3.34 b	5.22 a	ns
E-L36	7.88	8.74	7.90	8.71	**
E-L37	20.37 b	32.55 a	29.39 a	23.53 b	ns
E-L38	43.76	31.13	31.57	43.32	*

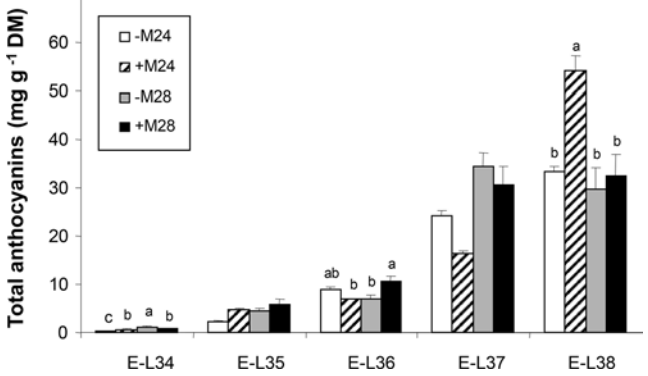


Fig. 2. Experiment 1: evolution of total anthocyanins recorded during berry ripening in fruit-bearing cuttings of Tempranillo (CL-1048) inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M) and grown at 24/14 °C (24) or 28/18 °C (28) (day/night) temperatures during the berry ripening. Values represent means ± SE (n = 4). Within each phenological stage, a two-way ANOVA analysis was performed to evaluate the effects of temperature (T), mycorrhizal inoculation (M) and their interaction. ns, * and ** indicate non-significance or significance at 5% or at 1% probability levels, respectively. When the interaction between the main factors 'temperature, T' and 'mycorrhizal inoculation, M´ was significant, histograms with different letters indicate significant differences according to Duncan test (P ≤ 0.05).

3.1. Climate change scenario: effects of warming temperature

The results showed that mycorrhizal colonization of CL-1048 Tempranillo reached high values (to ca. 40%) and that this percentage increased significantly at 28/18 °C (Table 1). Several authors have reported that elevated temperature increased the abundance of mycorrhizas [61], mycorrhizal colonization and hyphal length [62] by enhancing carbon allocation of AMF and increasing phosphorus acquisition [63]. In contrast, Wilson et al. [64] reported that increased temperature diminished mycorrhizal colonization, and this effect was consistent across the Mediterranean climate gradient. These inconsistencies could be due to the role that AMF ultimately play in the alteration of the carbon storage capacity of soils and could be dependent on changes in the structure of the AMF network and the flux of labile photosynthates from plants to the fungus [65].

In the current study, no significant effect of AMF on phenolic content was found (Table 1), which contrasts with results of Torres et al. [24]. These discrepancies could be due to differences in the rates of AMF colonization in the former study, which were much lower than those presented here. Thus, the high carbon cost of symbiosis maintenance could have resulted in limited carbon available for phenolic biosynthesis under elevated temperatures [61,65]. Moreover, the type of mycorrhizal inocula could have also exerted an influence because Torres et al. [24] used a commercial inoculum derived from an in vitro culture of *Rhizophagus intraradices*. In the present study, grapevines received a mixture of five AMF (see Material and Methods section), which reinforces the idea that it may be useful to identify the AMF inoculants most suitable for a given variety or cultivar in a given environment [66]. On the other hand, the temperature was the main factor reducing the content of phenolic substances in berries (Table 1). Similarly, other studies have reported significant reductions in phenolic content in berries at high temperatures [10], which have recently been linked to increased peroxidase activity under these conditions [12,67].

At 28/18 °C berries of CL-1048 reached berry maturity (estimated as total soluble sugars) 20 days earlier than plants exposed to 24/14 °C

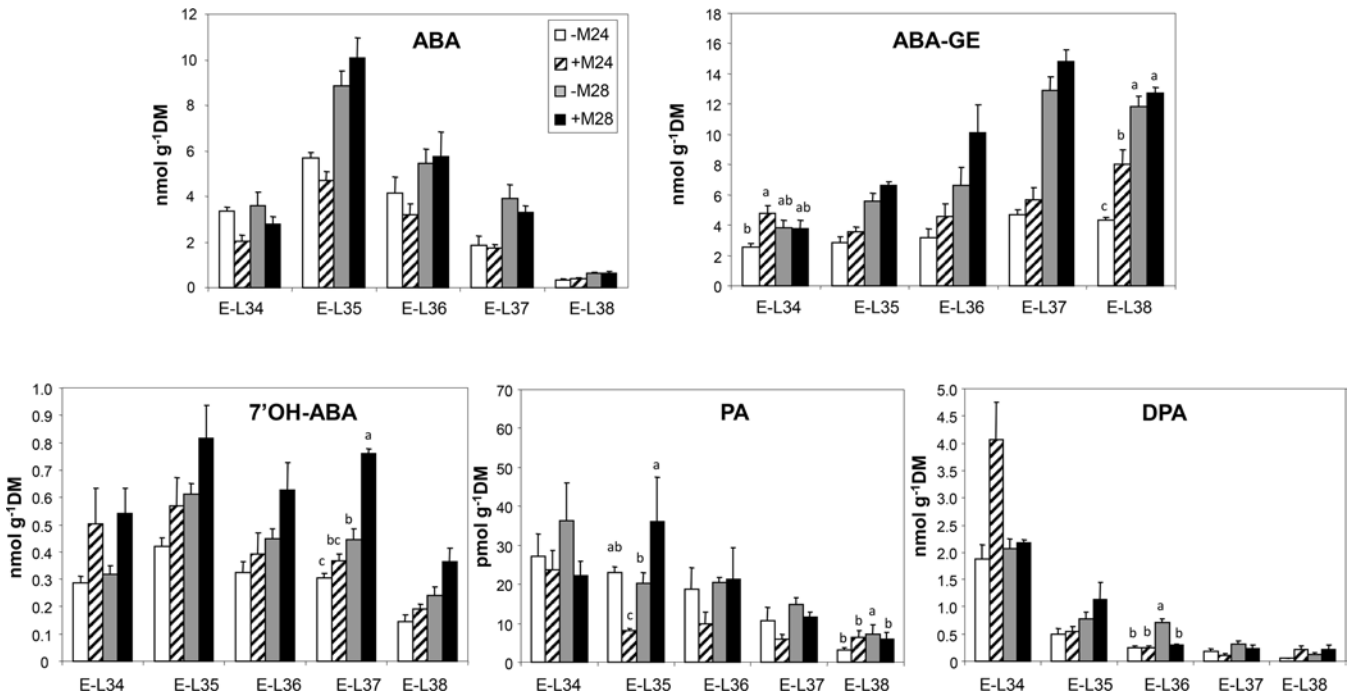


Fig. 3. Experiment 1: evolution of berry ABA and its catabolites measured in fruit-bearing cuttings of Tempranillo (CL-1048) inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M) and grown at 24/14 °C (24) or 28/18 °C (28) (day/night) temperatures during the berry ripening. Values represent means ± SE (n = 4). Within each phenological stage, when the interaction between the main factors 'temperature, T' and 'mycorrhizal inoculation, M´ was significant, histograms with different letters indicate significant differences according to Duncan test (P ≤ 0.05). *ABA*: abscisic acid, *ABA-GE*: abscisic acid glucosylester; *7'OH-ABA*: 7-hydroxy-ABA; *DPA*: dihydrophaseic acid; *PA*: phaseic acid.

Table 2
Experiment 1: main effects and their interactions on berry ABA and its catabolites quantified during ripening in fruiting cuttings from Tempranillo (CL-1048) inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M) and grown at 24/14 °C (24) or 28/18 °C (28) (day/ night) temperatures (T).

	Main effects				ANOVA		
	Temperature (T)		Mycorrhization (M)		T	M	T × M
	24	28	-M	+M			
ABA (nmol g^{-1} DM)							
E-L 34	2.7	3.0	3.3 a	2.4 b	ns	*	ns
E-L 35	5.2 b	9.5 a	7.3	7.4	***	ns	ns
E-L 36	3.7 b	5.6 a	4.7	4.5	*	ns	ns
E-L 37	1.8 b	3.6 a	2.9	2.5	***	ns	ns
E-L 38	0.4 b	0.6 a	0.5	0.5	**	ns	ns
ABA-GE (nmol g^{-1} DM)							
E-L 34	3.8	3.7	3.2	4.3	ns	ns	*
E-L 35	3.2 b	6.1 a	4.2 b	5.1 a	***	*	ns
E-L 36	3.9 b	8.4 a	4.9 b	7.4 a	**	*	ns
E-L 37	5.2 b	13.9 a	8.8 b	10.3 a	***	ns	ns
E-L 38	6.2	12.3	8.1	10.4	***	**	*
7'OH-ABA (nmol g^{-1} DM)							
E-L 34	0.39	0.43	0.30 b	0.52 a	ns	*	ns
E-L 35	0.50 b	0.71 a	0.52	0.59	**	ns	ns
E-L 36	0.36 b	0.54 a	0.39	0.51	**	ns	ns
E-L 37	0.34	0.60	0.37	0.56	***	***	***
E-L 38	0.17 b	0.30 a	0.19 b	0.28 a	**	*	ns
PA (pmol g^{-1} DM)							
E-L 34	22.4	24.3	31.8 a	23.0 b	ns	*	ns
E-L 35	15.6	28.2	21.7	22.0	*	ns	**
E-L 36	12.8 b	20.1 a	19.3 a	13.7 b	*	*	ns
E-L 37	10.0	13.2	14.4 a	8.9 b	ns	*	ns
E-L 38	4.2	5.9	5.2	4.9	*	ns	**
DPA (nmol g^{-1} DM)							
E-L 34	2.97	2.12	1.97 b	3.13 a	ns	*	ns
E-L 35	0.52 b	0.95 a	0.63	0.84	*	ns	ns
E-L 36	0.25	0.50	0.47	0.27	***	***	***
E-L 37	0.14 b	0.27 a	0.24	0.17	*	ns	ns
E-L 38	0.13	0.17	0.08 b	0.22 a	ns	**	ns

Values represent means (n = 4) separated by Duncan's test (at $P \leq 0.05$). Within rows, means followed by different letters are significantly different as affected by the main factors temperature (24, 28), mycorrhization (+M, –M) and their interaction. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ns, not significant ($P > 0.05$). *ABA*: abscisic acid, *ABA-GE*: abscisic acid glucosylester; *7'OH-ABA*: 7-hydroxy-ABA; *DPA*: dihydrophaseic acid; *PA*: phaseic acid; DM: dry matter.

Table 3
Experiment 2: percentage of mycorrhizal colonization and total phenolics recorded at the harvest of fruit-bearing cuttings of Tempranillo (CL-1089) inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M), grown at 24/14 °C (24) or 28/18 °C (28) (day/ night) temperatures (T) and subjected to different irrigation (I) regimes (FI: full irrigation; LD: late season water deficit).

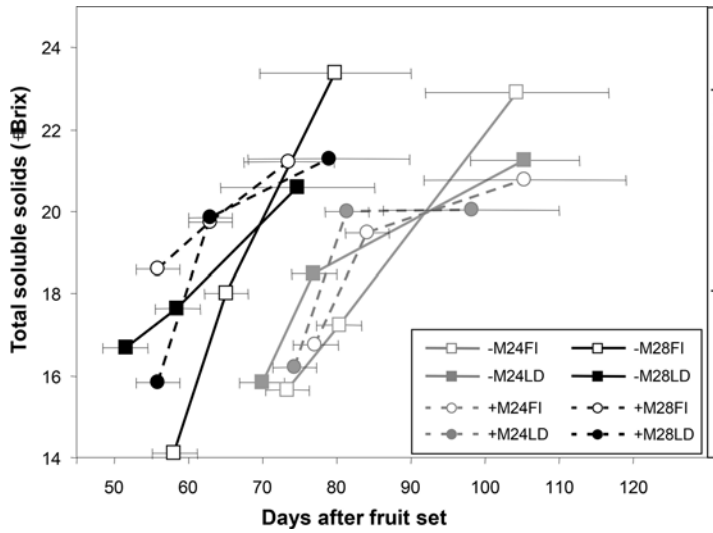
	Mycorrhizal colonization (%)	Soluble phenolic substances (mg g^{-1} DM)
Treatments		
-M24-FI	–	41.9
-M24-LD	–	40.6
+M24-FI	65.7	39.8
+M24-LD	46.3	43.4
-M28-FI	–	37.1
-M28-LD	–	39.5
+M28-FI	67.0	38.2
+M28-LD	83.7	48.5
Main effects		
Temperature (T)		
24	56.0 b	41.4
28	75.3 a	40.8
Mycorrhization (M)		
-M	–	39.8
+M	–	42.5
Irrigation (I)		
FI	66.3	39.3
LD	65.0	43.0
ANOVA		
T × M × I	–	ns

Values represent means (n = 4) separated by Duncan's test (at $P \leq 0.05$). Within columns, means followed by different letters are significantly different as affected by the main factors temperature (24, 28), mycorrhization (+M, −M), irrigation (FI, LD) and their interactions. ns, not significant ($P > 0.05$). DM indicates dry matter.

(Fig. 1), which agrees with the known effect of temperature to accelerate grapevine phenology [68]. AMF inoculation dampened the effect of elevated temperature, with berry sugars being similar under both temperature conditions (T × M, $P \leq 0.05$) (Fig. 1). Nevertheless, in –M28 plants, the acceleration of phenology led to higher levels of sugars than those obtained at 24/14 °C. Furthermore, the pattern of anthocyanin accumulation throughout berry ripening was significantly modified by AMF inoculation and/or temperature (Fig. 2). The study of the main factors (temperature and mycorrhization) revealed that, while AMF induced the accumulation of anthocyanins in grapes at veraison (E–L 35 stage), elevated temperature favoured the accumulation of these phenolic compounds at the E–L 37 stage. At harvest (E–L 38), however, the highest levels of anthocyanins were found in berries from + M plants cultivated at 24/14 °C. Mycorrhizal symbiosis also increased the content of anthocyanins in strawberry fruits [69,70], which has been attributed to the up-regulation of some genes responsible for phenylpropanoid biosynthesis, such as phenylalanine ammonia lyase (PAL), a key enzyme involved in the synthesis of many phenolic compounds [71].

The influence that AMF symbiosis and temperature exerted on ABA metabolism was examined profiling ABA and its catabolites throughout berry ripening (Fig. 3). The endogenous free ABA content is determined by the dynamic balance between biosynthesis and catabolism [35]. In our study, the concentrations of ABA and its catabolites were assessed at the same time as sugars and anthocyanins. As expected, free ABA content peaked at veraison (E–L 35 stage) and decreased thereafter in all treatments (Fig. 3). The concentrations of ABA-GE increased during berry ripening, reaching a maximum content at maturity, whereas the concentrations of 7'OH-ABA were reduced at the E–L 38 stage and PA and DPA continuously diminished from the E–L 34 to E–L 38 stages. Current research is focusing on ABA catabolites, which have been recently highlighted as key molecules in grapevine development [34] and in its physiological responses to environmental stresses [37–39]. The ABA-GE acts as a reservoir of ABA and controls its concentration via the release of ABA by β–glucosidase. Moreover, ABA can be catabolized by hydroxylation at the positions 7′ and 8′, which produces 7′OH-ABA or PA and DPA, respectively. Our results showed that mycorrhizal inoculation was the main factor modulating levels of ABA derivatives by increasing ABA-GE, 7´OH-ABA and DPA and by decreasing PA content in most of the stages studied (Table 2). Temperature also modulated the free ABA content of berries, since warm temperatures resulted in increased concentrations of ABA in the most stages analysed. This could be related to the up-regulation of NCED genes under these conditions suggesting the participation of ABA in berry acclimation responses to high temperature [28]. Similarly, temperature also contributed to increasing levels of ABA-GE and 7´OH-ABA, and to a lesser extent, PA and DPA (Table 2). At the late stages of berry maturation (E–L 37 and E–L 38) both temperature and AMF influenced ABA metabolism, which led to higher ABA-GE (T × M, $P \leq 0.05$) and 7′OH-ABA (T × M, $P \leq 0.001$) and lowers PA (T × M, $P \leq 0.01$) in the + M28 treatment (Table 2).

It has been reported that in grapes ABA hydroxylation at the 8´position predominates over the 7´position [34]. In contrast, our results suggest that, at the end of berry ripening (E–L37 and E–L 38



°Brix	24	28	-M	+M	FI	LD	ANOVA T×M×I
	Main effects						
E-L36	16.1	16.3	15.6	16.9	-	-	ns
E-L37	18.8	18.8	17.9	19.8	18.6	19.0	ns
E-L38	21.2	21.6	22.0	20.8	22.1	20.8	ns

Days	24	28	-M	+M	FI	LD	ANOVA T×M×I
	Main effects						
E-L36	73.8 a	55.5 b	63.3	65.9	-	-	ns
E-L37	80.8 a	62.5 b	70.3	72.9	73.2	70.0	ns
E-L38	103.4 a	76.8 b	91.1	89.1	90.8	89.4	ns

Fig. 4. Experiment 2: evolution of total soluble solids (°Brix) recorded in fruit-bearing cuttings of Tempranillo (CL-1089) inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M), grown at 24/14 °C (24) or 28/18 °C (28) (day/night) temperatures and subjected to different irrigation regimes (FI, full irrigation; LD, late season deficit irrigation) during berry ripening. Values represent means ± SE (n = 4). Within each phenological stage, a three-way ANOVA analysis was performed to evaluate the effects of temperature (T), mycorrhizal inoculation (M), irrigation (I) and their interactions. ns indicate non-significance.

stages), AMF inoculation under warming temperatures promoted ABA catabolism by means of 7´OH-ABA (Fig. 3). Other authors have indicated that 7´OH-ABA may be active in some hormonal processes, showing ABA-like activity and up-regulating secondary metabolism-related genes [72]. Although little is known about the role of this catabolite in berry ripening, Owen et al. [73] suggested that the increase in some ABA metabolites could make ABA unnecessary. This idea could help to explain some effects of AMF inoculation such as the enhancement of anthocyanin content related to lower levels of the inactive conjugated (ABA-GE) in +M24 treatment at maturity (E–L 38 stage) or the advancement of anthocyanin biosynthesis after veraison (E–L 37 stage) related to higher 7′OH-ABA in +M28 plants (Figs. 2 and 3).

3.2. Climate change scenario: combined effects of deficit irrigation and warming temperature

Mycorrhizal colonization of Tempranillo CL-1089 reached high values (ca. 60%) at 24/14 °C and this percentage increased significantly at 28/18 °C, attaining values up to 75%, (Table 3) in accordance with references discussed above. On the other hand, total phenolic content in berries was not significantly modified by any of the three factors applied (AMF, temperature or irrigation regime), corroborating that CL-1089 could be a good candidate to cope with global warming due to its ability to maintain certain fruit quality traits under these conditions [24].

As indicated in experiment 1, temperature was the main factor accelerating berry maturity (estimated as total soluble sugars) regardless of the AMF inoculation or irrigation level applied (Fig. 4). In contrast,

	24	28	-M	+M	FI	LD	ANOVA T×M×I
			Main effects				
E-L36	19.05 a	12.90 b	14.04	17.90	-	-	-
E-L37	35.99	31.73	29.72	38.00	36.55	31.17	***
E-L38	37.56	45.02	38.52	44.06	40.10	42.48	**

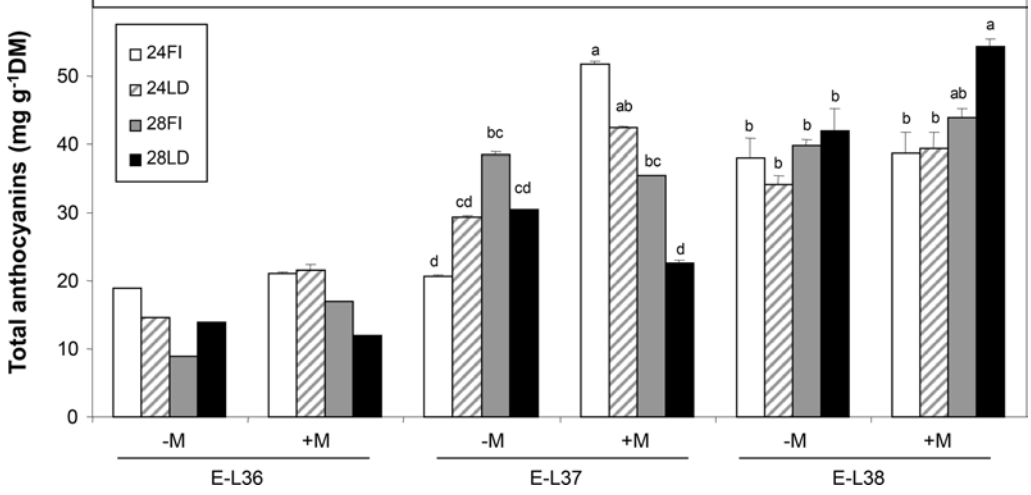


Fig. 5. Experiment 2: evolution of total anthocyanins measured in fruit-bearing cuttings of Tempranillo (CL-1089) inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M), grown at 24/14 °C (24) or 28/18 °C (28) (day/night) temperatures and subjected to different irrigation regimes (FI, full irrigation; LD, late season deficit irrigation) during the berry ripening. Values represent means ± SE (n = 4). Within each phenological stage, a three-way ANOVA analysis was performed to evaluate the effects of temperature (T), mycorrhizal inoculation (M), irrigation (I) and their interactions. ** and *** indicate significance at 1% or at 0.1% probability levels, respectively. When the interaction between the main factors 'temperature, T', 'mycorrhizal inoculation, M´ and ´irrigation, I´ was significant, histograms with different letters indicate significant differences according to Duncan test (P ≤ 0.05).

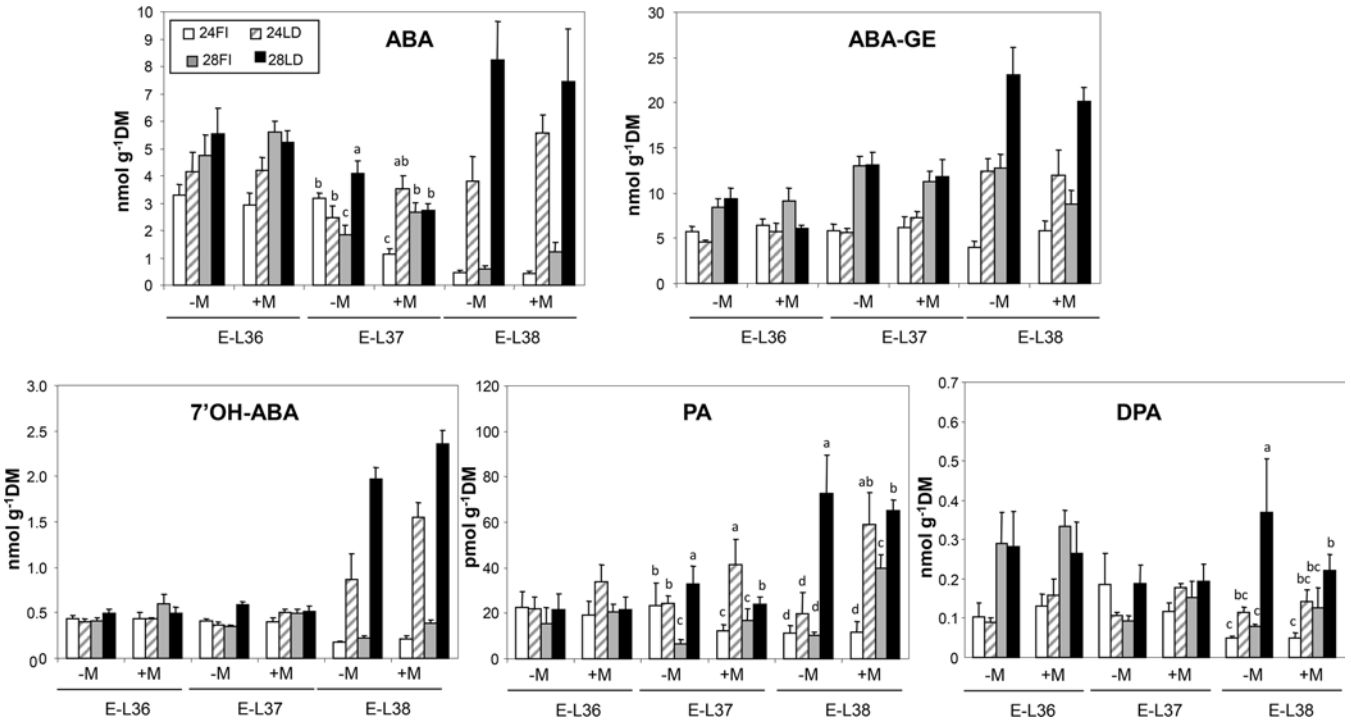


Fig. 6. Experiment 2: evolution of berry ABA and its catabolites recorded in fruit-bearing cuttings of Tempranillo (CL-1089) inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M), grown at 24/14 °C (24) or 28/18 °C (28) (day/night) temperatures and subjected to different irrigation regimes (FI, full irrigation; LD, late season deficit irrigation) during the berry ripening. Values represent means ± SE (n = 4). Within each phenological stage, when the interaction between the main factors 'temperature, T', 'mycorrhizal inoculation, M´ and ´irrigation, I´ was significant, histograms with different letters indicate significant differences according to Duncan test (P ≤ 0.05). *ABA*: abscisic acid, *ABA-GE*: abscisic acid glucosylester; *7′OH-ABA*: 7-hydroxy-ABA; *DPA*: dihydrophaseic acid; *PA*: phaseic acid.

the anthocyanin content was mostly affected by the three factors applied (T × M × I, P ≤ 0.001 and P ≤ 0.01 for E–L 37 and E–L 38, respectively) (Fig. 5). Thus, under the most stressful climate change conditions (LD and 28/18 °C) anthocyanin accumulation was significantly improved at the E–L 38 stage, especially in AMF inoculated plants, in agreement with preceding results obtained in this clone [25]. These observations could be explained by the ability of AMF symbiosis to stimulate the production of secondary metabolites in plants [22], together with the suitability of a post-veraison water deficit (LD) schedule to improve anthocyanin content [13,15,46].

The influence of AMF symbiosis, temperature and deficit irrigation on ABA metabolism was assessed profiling ABA and its catabolites from the end of veraison until berry maturity (Fig. 6). Our results showed that under elevated temperature AMF inoculation reduced ABA-GE concentrations in all phenological stages studied and DPA concentration in the E–L 38 stage, (T × M, P ≤ 0.05) (Table 4). Moreover, at berry maturity, AMF inoculation contributed to increasing 7´OH-ABA concentrations under LD conditions (M × I, P ≤ 0.05). Recent studies indicated that the ABA catabolism/conjugation processes play an important role under environmental constraints. Thus, Balint and Reynolds [37,38] reported that ABA was mainly catabolized by conjugation to form ABA-GE in plants subjected to water deficit, which agrees with the high rates of ABA-GE detected in the LD treatment, especially under elevated temperature (Fig. 6).

It has been reported that in grape berries, the patterns of mRNA expression associated with ABA metabolism were altered under water deficit that, in turn, this could modify the endogenous ABA content of berry [18,31,44,74]. Similarly, our results showed that the imposition of LD altered the pattern of ABA accumulation, consisting of a significant prolongation of ABA production over time (Fig. 6). In a previous study we have reported that in the LD treatment ABA accumulation lasted until to the end of veraison [46]. The data presented here show that under LD, ABA accumulation was prolonged to berry maturity (E–L 38 stage), especially under warming conditions. However, at the E–L 37 stage ABA was modulated by the three factors applied that led to lower ABA concentrations in the berries of +M plants subjected to 28LD (T × M × I, P ≤ 0.001) (Table 4).

Overall, under the most stressful climate change conditions (LD and 28/18 °C) ABA catabolism was seriously altered by AMF inoculation. Indeed, at the E–L 38 stage, +M plants showed a preferential pathway of ABA degradation to 7´OH-ABA that was modulated by all the factors applied (T × I, P ≤ 0.001 and M × I, P ≤ 0.05) (Table 4). However, in –M plants ABA catabolism seemed to occur mainly by means of degradation to PA and DPA (8′ hydroxilation pathway), as shown by the higher PA and DPA concentrations in the 28LD treatment at maturity (T × M × I, P ≤ 0.001 and P ≤ 0.05, respectively). Similar findings were obtained in non-mycorrhizal Tempranillo plants subjected to a combination of water deficit and high temperature and were related to lower anthocyanin content at maturity [39]. Furthermore, the observed differences in ABA catabolism pathways between –M and +M plants in our study could explain why berries in the –M28LD treatment reached lower anthocyanins than those of +M28LD (Fig. 5). Since phytohormonal homeostasis in the plant host is also modulated by AMF symbiosis [40,42,43], our study suggests that the observed changes in ABA metabolism under climate change conditions could contribute to explain the positive effects of AMF inoculation of Tempranillo on berry anthocyanins. Although this study does not provide information on the contents of minerals in grapes, AMF may exert a positive effect by increasing the uptake of some macro (mainly phosphorus) and micronutrients (such as iron, copper, manganese or zinc) which are accumulated in berries during growth and ripening [75].

4. Conclusions

The main findings of this study showed that ABA catabolism/conjugation throughout berry development was modified by AMF inoculation and by the climate change conditions and that 7´OH-ABA plays an important role in the anthocyanin content of Tempranillo

Table 4
Experiment 2: main effects and their interactions on berry ABA and its catabolites quantified during ripening in fruiting cuttings from Tempranillo (CL-1089) inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M), grown at 24/14 °C (24) or 28/18 °C (28) (day/ night) temperatures (T) and subjected to different irrigation (I) regimes (FI: full irrigation; LD: late season water deficit).

	Main effects						*Two factor interactions*												*ANOVA*						
	Temperature (T)		Mycorrhization (M)		Irrigation (I)														T	M	I	T × M	T × I	M × I	T × M × I
	24	28	-M	+M	FI	LD	-M24	+M24	-M28	+M28	24FI	24LD	28FI	28LD	-MFI	-MLD	+MFI	+MLD							
ABA (nmol g^{-1} DM)																									
E-L36	3.6 b	5.3 a	4.3	4.5	–	–	3.7	3.6	5.1	5.4	–	–	–	–	–	–	–	–	***	ns	–	ns	–	–	–
E-L37	2.6	2.8	2.9	2.5	2.2	3.2	2.8	2.3	3.0	2.7	2.1	3.0	2.2	3.4	2.5	3.2	1.9	3.1	ns	ns	***	ns	ns	ns	***
E-L38	2.6 b	4.4 a	3.2	3.7	0.7 b	6.3 a	2.1	3.0	4.4	4.3	0.4	4.7	0.9	7.8	0.5	6.0	0.8	6.5	**	ns	***	ns	ns	ns	ns
ABA-GE (nmol g^{-1} DM)																									
E-L36	5.5	8.2	7.0	6.7	–	–	5.1 c	5.8 bc	9.0 a	7.6 b	–	–	–	–	–	–	–	–	***	ns	–	*	–	–	–
E-L37	6.2	12.3	9.4	9.2	9.1	9.5	5.7 c	6.8 c	13.1 a	11.5 b	6.1	6.4	12.2	12.5	9.5	9.4	8.8	9.6	***	ns	ns	*	ns	ns	ns
E-L38	8.6	16.2	13.1	11.7	7.9	16.9	8.2 c	8.9 c	17.9 a	14.5 b	5.0	12.2	10.8	21.6	8.4	17.7	7.4	16.0	***	ns	***	*	ns	ns	ns
7′OH-ABA (nmol g^{-1} DM)																									
E-L36	0.42	0.50	0.43	0.49	–	–	0.42	0.43	0.45	0.54	–	–	–	–	–	–	–	–	ns	ns	–	ns	–	–	–
E-L37	0.42	0.48	0.43	0.47	0.41	0.49	0.38	0.45	0.47	0.50	0.40	0.43	0.42	0.55	0.38	0.47	0.44	0.50	ns	ns	ns	ns	ns	ns	ns
E-L38	0.70	1.23	0.81	1.12	0.25	1.69	0.52	0.88	1.10	1.37	0.20 c	1.21 b	0.30 c	2.16 a	0.20 c	1.42 b	0.30 c	1.95 a	***	**	***	ns	***	*	ns
PA (pmol g^{-1} DM)																									
E-L36	24.3	19.8	20.4	23.7	–	–	22.3	26.4	18.5	21.1	–	–	–	–	–	–	–	–	ns	ns	–	ns	–	–	–
E-L37	25.4	20.0	21.8	23.6	14.8	30.6	24.0	26.8	19.7	20.4	17.8	33.0	11.8	28.3	15.1	28.6	14.5	32.7	ns	ns	***	ns	ns	ns	***
E-L38	25.3	47.1	28.4	44.0	18.3	54.1	15.3	35.4	41.6	52.5	11.5	39.3	25.2	68.9	10.7	46.2	26.0	62.0	***	**	***	ns	ns	ns	***
DPA (nmol g^{-1} DM)																									
E-L36	0.12 b	0.29 a	0.19	0.22	–	–	0.10	0.15	0.29	0.30	–	–	–	–	–	–	–	–	***	ns	–	ns	–	–	–
E-L37	0.15	0.16	0.14	0.16	0.14	0.17	0.15	0.15	0.14	0.17	0.15	0.14	0.12	0.19	0.14	0.15	0.13	0.19	ns	ns	ns	ns	ns	ns	ns
E-L38	0.09 b	0.20 a	0.15	0.13	0.08 b	0.21 a	0.08 c	0.10 c	0.23 a	0.17 b	0.05	0.13	0.10	0.30	0.06	0.24	0.09	0.18	***	ns	***	*	ns	ns	*

Values represent means (n = 4) separated by Duncan's test (at $P \leq 0.05$). Within columns, means followed by different letters are significantly different as affected by the main factors temperature (24, 28), mycorrhization (+M, –M), irrigation (FI, LD) and their interactions. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ns, not significant ($P > 0.05$). *ABA*: abscisic acid, *ABA-GE*: abscisic acid glucosylester; *7′OH-ABA*: 7-hydroxy-ABA; *DPA*: dihydrophaseic acid; *PA*: phaseic acid.

berries. Thus, under elevated temperature, AMF inoculation contributed to increase berry anthocyanins and modulated ABA metabolism, which led to higher ABA-GE and 7′OH-ABA and lower PA concentrations in comparison with those in fruits of –M plants. Under the most stressful climate change conditions (elevated temperature and deficit irrigation) AMF-inoculated plants reached higher berry anthocyanins and evidenced some modifications in berry ABA catabolism, leading to increased ABA hydroxylation at the position 7′ in detriment of position 8′. Our findings provide an explanation of the ability of AMF to maintain and/or improve berry characteristics under future climatic conditions. To our knowledge, data presented in this study offer the first evidence on the implication of mycorrhizal symbiosis on grape ABA metabolism and how changes induced by AMF could affect some traits which determine the quality of grapes. Moreover, these results provide information on the role that AMF may play under future conditions of climate change. Several authors have studied the mycorrhizal communities associated with grapevines in the field. Results obtained in vineyards from USA, Italy, France or Central Europe subjected to agricultural practices such as high fertilizer inputs, tillage, weed control or pest management [76–79], have shown reduced diversity of AMF in comparison with that found in the rhizosphere of European wild grapevine [19]. Evidence on the role that AMF may play on berry hormonal status and thus on berry ripening in different environmental scenarios reinforces the need to protect the natural mycorrhizal fungal communities present in vineyards.

Acknowledgements

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.plantsci.2018.06.009.

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